Title of the Invention

OVULATION-PERIOD-DETECTING REAGENTS AND THE USE THEREOF

Field of the Invention

The present invention relates to a humoral detecting reagent, especially to an ovulation-period-detecting reagent and the use thereof.

Background of the Invention

Nowadays, most fertile women use medical contraception and instrumental contraception. But, most of them suffer a drug by-reaction from medial contraception, and ordinary contraceptive medicines have drawbacks of long period of medicine taking and being liable to be forgotten. Although instrumental conception, such as conception via the loop, has its advantages, the physical malaise to women is also obvious.

In addition to the above-mentioned contraception methods, women usually use the rhythm method, i.e. via calculating their ovulation period. The biggest drawback of this method lies in the fact that the period of ovulation cannot be calculated accurately, and the calculation error would be even bigger if the menstrual cycle is irregular. In this sense, the believed safe rhythm method is not safe.

Since a woman's temperature changes regularly during the period of ovulation, many people predict the period of ovulation by taking the body temperature every day. Obviously, such method is very complicated and tedious.

The inventor has found that before and after the period of ovulation, the content of peroxidase in the vaginal secretion changes remarkably and the peroxidase may catalyze a color reaction between certain substances and hydrogen peroxide. Based on this principle, this invention provides a simple, convenient and reliable method to detect the period of ovulation, thus solving the existing technical problems in the prior art.

Summary of the Invention

This invention provides an ovulation-period-detecting reagent, comprising:

Sub A1 Component A, comprising an aqueous solution of a substance conducting a color reaction with hydrogen peroxide; and

Component B, an aqueous solution of hydrogen peroxide.

In said Component A, the content of said substance may be 1-10% (by weight), and the content of hydrogen peroxide in Component B may be 1-10% (by weight).

Of the reagent according to this invention, said Component A may further contain a stabilizing agent.

This present invention also provides a kit for detecting the period of ovulation, containing Component A, Component B, a transparent container and cotton sticks. Said Component A comprises 1-10% (by weight) of a substance which can conduct a color reaction with hydrogen peroxide; and said Component B is 1-10% (by weight) aqueous solution of hydrogen dioxide.

This invention is also to provide a method to use said detecting reagent, which comprises mixing said Component A with said Component B, and putting a secretion from the vaginal of the detected woman into the resultant solution, and checking whether or not a color reaction takes place.

Detailed Description of the Preferred Embodiments

According to this invention, the ovulation-detecting reagent comprises a Component A and a Component B, and said Component A further containing a stabilizing agent.

The substance involving in said Component A, which can conduct a color reaction with hydrogen peroxide, is usually selected from benzidine compounds, such as benzidine, tetramethyl benzidine, diaminobenzidine, o-tolidine, o-dianisidine, and the like. Inorganic

salts of said benzidine compounds such as hydrochloride and sulfate thereof may also be used as active substances in said Component A.

Besides benzidine compounds, those which can conduct a color reaction with hydrogen peroxide may also be used in this invention. To be specific, said substances include 3-amino-9-ethylcarbazole, 4-methoxy-α-naphthol, o-phenylenediamine, 5-aminosalicylic acid, 2,2-azo-di(3-ethyl-benzothiazoline-6-sulfonate), pyrogallol, o-methoxyphenol, and the like.

Although those stabilizing agents commonly used in this field can be used as the stabilizer in the Component A, sodium benzoate is preferably used in this invention.

This invention further provides a kit for detecting the period of ovulation, which contains a Component A, a Component B, a transparent container and cotton sticks.

In said kit, said Component A and Component B are the same as the above. The content of substances in said Component A (by weight) may be of 1-10%, preferably 5-8%; and the content of hydrogen dioxide in said Component B (by weight) can be of 1-10%, preferably of 4-8%. The proportion between the Component A and the Component B must be kept at a level to satisfy the minimum requirement of the color reaction. Said proportion depends on the specific substance to be used in the Component A, and the appropriate ratio between them (by volume) may be 10-20:1.

In practical use, Component A and Component B are mixed in the transparent container. Then the vaginal secretion collected with a cotton stick is put into the solution to check whether a color reaction takes place, thus determining whether the detected woman is in the period of ovulation.

The principle of this invention is briefly described as follows:

Before and after the period of ovulation, the critical point of peroxidase in the vaginal secretion is usually kept at 25 x 10^{-3}-25 x 10^{-5}u/ml. Within 3-4 days before and after ovulation, the content of peroxidase in the vaginal secretion decreases obviously below the said critical point. Outside the period of ovulation, however, the content of peroxidase in the vaginal secretion is obviously above the critical point. Therefore, it can be determined whether a woman is in the period of ovulation through measuring the content of peroxidase in her vaginal secretion.

The content of said peroxidase is the very content required for catalyzing a color reaction between hydrogen peroxide and certain chemical compounds. Without peroxidase, it would take dozens of minutes or even longer time to finish the color reaction between hydrogen peroxide and the chemical compounds. As a catalyst, however, said content of the peroxidase can make the color reaction finished within a couple of seconds. The present invention is just based on this theory. After the vaginal secretion from a tested woman is put into the mixed solution of Component A and Component B, if the color reaction takes place in the several seconds, it shows that the

content of peroxidase in her vaginal secretion is above the critical level. That is, she is in the safe period. Otherwise, she is in the period of ovulation.

The kit for detecting the period of ovulation is specifically used in the following way:

Component A and Component B are mixed in the ratio of 10-20:1(by volume) and the mixed solution is put into the transparent container. Then, a cotton stick is used to collect some vaginal secretion and the stick is dipped into the prepared reagent. If the color of the reagent does not change, it means that the tested woman is in the period of ovulation. On the contrary, the change of the reagent color indicates that the tested woman in the safe period.

The following clinical experiments have been conducted to test the sensibility and stability of the reagent according to the invention.

1) Experiment of sensibility:

Test 1:

A 1% solution of sodium benzoate (by weight) was added to a 5% solution of tetramethyl benzidine (hereinafter referred to as: TMB) (by weight) to obtain the Component A, and a 2% aqueous solution of hydrogen peroxide was used to prepare the Component B. Then, three portions of balance samples were made by mixing 1 ml of the Component A with 0.05ml of the Component B. To the balance samples was added 50ul of the standard solution with 25 x 10^3u/ml peroxidase, respectively. Other three samples without peroxidase were made as control samples. The result of the experiment was shown in Table 1.

Table 1

Items	Tested Samples			Control Samples		
	1	2	3	1	2	3
Component A (TMB) +Component B (H_2O_2)	A+B	A+B	A+B	A+B	A+B	A+B
Reaction Time (second)	15	15	15	15	15	15
Color Displayed	blue	blue	blue	colorless	colorless	colorless
Sensibility (%)	100%	100%	100%	100%	100%	100%

Test 2:

A 1% solution of sodium benzoate (by weight) was added to a 2% solution of 3-amino-9-ethylcarbazole (hereinafter referred to as: AEC) (by weight) to obtain Component A, and a 2% aqueous solution of hydrogen peroxide (H_2O_2) was used to make Component B. Then, three portions of balance samples were made by mixing 1ml of the Component A with 0.05 ml of the Component B. To the balance samples was added 50ul of the standard solution with 25 x 10^3u/ml peroxidase, respectively. Other three samples without peroxidase were made as control samples. The result of the experiment was shown in Table 1.

Table 2

Items	Experiment samples			Control Samples		
	1	2	3	1	2	3
Component A (AEC) + Component B (H_2O_2)	A+B	A+B	A+B	A+B	A+B	A+B
Reaction Time (second)	30	30	30	30	30	30
Color Displayed	red	red	red	yellow*	yellow*	yellow*
Sensibility (%)	100%	100%	100%	100%	100%	100%

* The control sample itself showed yellow color.

The experiment results in Test 1 and Test 2 show that the sensibility reaches 100% within 15-30 seconds.

2) Experiment of stability:

Tetramethyl benzidine was used to prepare Component A of 5% concentration (by weight). To said Component A was added a 0.01% solution of sodium benzoate as a control sample. The samples were kept still for 6 to 30 months to compare their sensibility. The result was shown in Table 3.

Table 3

Composition of Component A	Valid Time	Sensibility
TMB	6 months	100%
TMB	6-12 months	50%
TMB +Sodium Benzoate	24-30 months	100%

The above result indicates that the stability of the Component A comprising sodium benzoate was increased from 6 months to above 24 months.

3) Clinical Studies:

After two years clinical test of this invention in the hospitals throughout China, the following results have been obtained:

Table 4

Hospitals	Person-times of ovulation period test	Conformity percentage	Person-times of natural contraception	Success percentage
Beijing Hospital for Gynecology and Obstetrics	34	100%	66	100%
Beijing Hospital	0	0	50	100%
Second Hospital Attached to Nanjing Medical University	40	100%	10	100%
Jinan Municipal Hospital for Women and Children	30	94%	20	100%
5th Hospital of Wuxi	75%	100%	25	100%
4th Hospital of Wuxi	35	100%	15	100%
Total	241	98.8%	186	100%

The results of clinical experiment showed that 98.8% of the 214 cases of ovulation period test were successful.

In 186 cases of natural contraception, all the target women used the contraception method of the safe period without any other contraceptive measures for 6-24 months in succession and no case of pregnancy occurred, making the success rate of natural contraception 100%.

The invention will be described in detail with the following examples.

Example 1

5kg of tetramethyl benzidine (TMB) was added to 95kg of medical water, then, 1kg of 1% solution of sodium benzoate was added to the former solution before being stirred at the speed of 10 rounds/min for 1.0 hour. The stirred solution was kept for 8 hours, and the precipitate was removed to collect the supernatant as Component A in a 20ml bottle. Hydrogen peroxide and medical water were used to obtain a 1% aqueous solution of hydrogen peroxide as Component B, which was bottled with 2ml each. Bottles of Component A and Component B together with cotton sticks and a transparent container were packed to obtain a kit of the invention.

Example 2

1kg of the 3-amino-9-ethylcarbazole (AEC) was mixed with 99kg of medical water to obtain a 1% solution. To the solution was added 1.5 kg of 1.5% solution of sodium benzoate. The resultant solution was stirred at the speed of 12 rounds/min for 0.8 hour and then was kept still for 9 hours. After the precipitate was removed, the supernatant was collected as Component A, which was bottled with 30ml each. Hydrogen peroxide and medial water were used to prepare a 5% (by weight) solution to obtain Component

B, which was bottled with 2ml each. Bottles of Component A and Component B together with cotton sticks and a transparent container were packed to obtain a kit of the invention.

Example 3

10kg of 4-methoxy-α-naphthol was mixed with 90kg of medical water to obtain a 10% solution. To the solution was added 2 kg of a 2% solution of sodium benzoate. The resultant solution was stirred at the speed of 15 rounds/min for 0.5 hour and then was kept still for 10 hours. After the precipitate was removed, the supernatant was collected as Component A, which was bottled with 40ml each. Hydrogen peroxide and medial water were used to prepare a 10% (by weight) solution to obtain Component B, which was bottled with 2ml each. Bottles of Component A and Component B together with cotton sticks and a transparent container were packed to obtain a kit of the invention.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention, which is defined by the appended claims.